NUCLEAR PHENOMENA OF SEXUAL REPRODUCTION IN GYMNOSPERMS[1]

CHARLES J. CHAMBERLAIN

UNIVERSITY OF CHICAGO

To the cytologist the most interesting phases of a plant's life history are fertilization and the reduction of chromosomes, processes which initiate the sporophyte and gametophyte generations and which are of the utmost importance in any cytological theories of heredity.

We shall not attempt to define fertilization, but shall simply state that, in our opinion, the process is essentially uniform from its first appearance in the fusion of equal gametes in the lower algæ, up to the heterogamy of the angiosperms and, in our opinion, this fusion of gametes, whether they be the equal gametes of the lower algæ or the unequal gametes of the higher plants, always initiates a sporophytic phase in the life history, a phase which normally continues until the reduction of chromosomes brings it to a close and initiates the gametophytic phase.

The gymnosperms have as yet only a single well-established case of apogamy and not even a single case of apospory, and, consequently, their only mode of reproduction, aside from occasional budding, is that resulting from fertilization.

Since the significance of fertilization becomes more intelligible with increasing knowledge of the participating gametes, it is of prime importance to know the structure, evolution and behavior of the sperms and eggs.

SPERMATOGENESIS

The sperms of fossil gymnosperms are almost unknown, but it is safe to say that Cycadofilicales and

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Cordaitales had swimming sperms, and further, that they had no pollen tubes, the pollen grains reaching the female gametophyte directly, then discharging their sperms about as in the living heterosporous Pteridophytes.

Definite knowledge of the structure of the sperm begins with the cycads, where the sperms are so large that they are easily visible to the naked eye. Broadly speaking, their development is like that of most pteridophytes. In the most thoroughly investigated fern, after the spermatogenous divisions have ceased, two blepharoplasts appear in each cell, which then divides so that each of the two resulting cells contains one nucleus and one blepharoplast. From the blepharoplast there is developed a more or less spiral band which gives rise to numerous cilia.

In gymnosperms, with the exception of *Microcycas, Cupressus* and occasionally, *Ceratozamia,* no spermatogenous divisions precede the formation of the cell which is to produce the pair of sperms, and in these three genera it is not known whether the blepharoplast appears any earlier than in the pteridophytes. I suspect that it does not, but it is certain that in the cycads and in *Ginkgo* two blepharoplasts always appear in the body cell which is to produce the pair of sperms, and while the blepharoplasts are at first very inconspicuous, they finally become larger than the nuclei of most angiosperms.

The mature sperm of the cycad consists of a very large nucleus surrounded by a thin layer of cytoplasm in which is imbedded the spiral band with its thousands of cilia. Compared with the sperms of the pteridophytes, the sperms of the cycads are immensely larger and much less numerous. It must be a fact of some significance that the living gymnosperms, with two or three exceptions, have only two sperms, for the production of sperms in pairs is universal from the liverworts to the orchids. Whether the production of sperms in pairs is associated with a separation of sexes is not

known. Objections to such a suggestion are easily raised, but the question seems worth investigation, especially since little is known of the behavior of the chromatin during the mitosis by which two sperms are produced from the body cell.

Except in the cycads and *Ginkgo,* there are no motile sperms in living gymnosperms, but, in our opinion, the transition is not so abrupt as some writers believe. The definitely organized male cells of such genera as *Sequoia* and *Thuja* look very much like the young sperms of a cycad immediately after the division of the body cell, the principal difference being the absence of the blepharoplast, which is such a conspicuous feature in the development of the sperms of cycads and *Ginkgo.* In regard to the several genera with well-organized male cells, the statement is made that there are no structures which could be interpreted as the vestiges of blepharoplasts, but the figures accompanying the various accounts are not convincing and it seems entirely possible that vestiges may yet be found.

According to all the accounts, either as expressed in the text or to be inferred from the figures, the body cell in Taxeæ, Taxodieæ and Cupresseæ gives rise directly to the male cells, there being no formation of sperms within sperm mother-cells. The accounts may be correct, but it must be remembered that a competent observer described just such a condition in one of the cycads, where it is now known that the sperms are formed within sperm mother-cells from which they are afterwards discharged. Among the Coniferales, the well-organized male cell is found in the Taxaceæ, Taxodieæ and Cupresseæ.

In the rest of the Coniferales, which means the Araucarieæ and Abieteæ, there are no organized male cells, but only male nuclei lying free in the cytoplasm of the body cell, and this cytoplasm not always sharply limited from that of the pollen tube. Accompanying these male nuclei there are often structures which might be interpreted as the vestiges of blepharoplasts. According to some observers, one of the male nuclei is smaller than the

other, possibly indicating the future elimination of the smaller nucleus.

That both the male cell of *Thuja* and the male nucleus of *Pinus* are descendants of swimming sperms is not to be doubted. The male cell of *Thuja* has lost its cilia and, perhaps, is no longer formed within a parent cell, while *Pinus* has gone further and no longer organizes a definite male cell. In this respect, the *Pinus* gametophyte is more widely separated from the ancestral form than is the gametophyte of *Thuja*.

In some genera, like *Torreya* and *Taxus*, the reduction has proceeded in another direction, two male cells being retained, but one of them having become much smaller than the other and having ceased to function, indicating its future elimination.

It is interesting to note that in Coniferales, with the exception of the Podocarpeæ, those forms with definitely organized male cells have no prothallial cells in the pollen grains, while those with the free male nuclei have retained more or less of the ancestral prothallium; *e. g.*, *Pinus* has retained the prothallial cells, but no longer organizes a definite male cell, while *Thuja* has retained the definitely organized male cell, but has lost the prothallial cells. And further, those genera which have retained the definitely organized male cell no longer organize a definite ventral canal cell, having lost the wall between the ventral canal nucleus and that of the egg, a step toward the complete elimination of even a ventral canal nucleus. Whether there are any causal relations among these reductions is not obvious, but it is interesting to note the correlation. If all evolutionary lines would only progress at the same pace, or if we could discover causal relations between the lines, it would facilitate the construction of phylogenies.

OOGENESIS

We have seen that in spermatogenesis the gymnosperms show a reduction series from the highly differentiated motile sperms of cycads to the free male nuclei

of *Pinus*. Oogenesis does not cover so great a range, for motile eggs are not found above the thallophytes. In its most primitive condition, the archegonium of the gymnosperms is more reduced than any found in pteridophytes, for there is no neck canal cell. An egg with a definite ventral canal cell, as in *Ginkgo* and *Pinus,* is the most primitive condition found in gymnosperms. Beyond this there is the elimination of the wall between the ventral canal nucleus and that of the egg, as in cycads and many conifers, a natural step in the elimination of the ventral canal nucleus, and in *Torreya,* even the nuclear division has probably failed to take place, so that the central cell functions directly as an egg. A still further reduction is found in *Tumboa* where the incomplete septation of the female gametophyte results in a failure to organize a definite egg; and finally, there is a complete suppression of any septation whatever, so that the egg is represented only by a nucleus with as little organization of cytoplasm about it as can be found in any angiosperm. Thus there has been a gradual reduction of the archegonium from a condition almost like that of the pteridophytes to the most extreme condition found in angiosperms.

Some might suggest that such reductions would have their natural termination in the elimination of all sexuality, with apogamy as the goal. In *Gnetum Ule* there seem to be instances of apogamy. In the cases reported as apogamy in *Pinus* there is the possibility of fertilization by the ventral canal nucleus. Personally, I prefer to regard apogamy as a specialized, unnatural phenomenon, and not as a condition toward which plants are moving.

The behavior of the chromatin in the final stages of both spermatogenesis and oogenesis in gymnosperms seems to be unique. At the formation of the ventral canal cell or ventral canal nucleus, the chromosomes are very small. The ventral nucleus or cell soon disintegrates, but the chromosomes of the egg nucleus form a spirem. From this point there is a period of develop-

ment for which we have no satisfactory account of the chromatin. The coarse reticulum of the egg nucleus is not chromatin, for most of it may remain after chromatin again becomes demonstrable. To say that the chromatin becomes dissolved in the linin or takes the form of coarse granules or nucleoli, which may or may not be chromatin at all, hardly solves the difficulty. That some of the so-called metaplasm has about the same position as the latest recognizable stages of the spirem, seems to be about all that can be said. It is certain that chromatin has not yet been traced from the telophase of the ventral canal cell mitosis to the resting egg nucleus with any such certainty as in the pteridophytes and angiosperms. The organization of the spirem from the dubious contents of this nucleus has not been traced in any satisfactory way. However, it is perfectly certain that a small and beautifully definite spirem finally appears.

Fertilization

To the cytologist, who is likely to attribute extreme importance to chromatin, these reduction series in the formation of eggs and sperms are very important, since the more there is eliminated from the structures taking part in fertilization, the more accurately can we determine what is essential and what only accessory.

Fertilization has been studied more thoroughly in *Pinus* than in any other gymnosperm. Here each archegonium has its own archegonial chamber and the pollen tube entering it necessarily discharges its contents into the one egg, the two male nuclei, together with the stalk and tube nuclei and also more or less cytoplasm and starch all entering the egg. One of the male nuclei comes into contact with the egg nucleus and the nuclear membranes at the point of contact break down, so that the chromatin of the two nuclei becomes surrounded by the membrane of the egg nucleus. A spirem is formed from the chromatin network of each of the sex nuclei and each spirem segments into 12 chromosomes, so that there are twenty-four chromosomes. These do not fuse with one

another, but become so mixed that the male and female chromosomes can not be distinguished. Each chromosome then splits and during the completion of the mitosis twenty-four chromosomes go to each pole to form the first two nuclei of the sporophyte generation. Consequently, during this process which we call fertilization, there has been no blending of the chromatin contributed by the two parents. Whether a real blending takes place as the two groups of chromosomes pass from the telophase of the first mitosis into the resting reticula of the daughter nuclei, is still undetermined. Personally, I am inclined to think that there is no blending, either at this early stage or later, but rather, that the chromatin contributions remain distinct throughout the life history. Whether there is, during the synapsis stage of the reduction division, sufficient fusion to impair the identity of the individual chromosomes, still remains to be demonstrated.

Although the chromosomes of the two groups become so mixed that they can not be distinguished, the well-known mechanism of mitosis makes it certain that one half of each chromosome contributed by the two parents will reach each of the two daughter nuclei resulting from the first division of the fertilized egg. The same mechanism of mitosis makes it very probable that this equal representation of the two parents will continue throughout the life history of the plant.

In all the genera which have been studied, more or less cytoplasm enters the egg with the male nucleus. In the cycads the entire sperm enters the egg and the cilia may continue to move after the sperm is within the cytoplasm of the egg, but the nucleus of the sperm soon slips out from the cytoplasmic sheath and advances toward the egg nucleus, leaving most or all of the cytoplasm in the upper part of the egg. In other forms, like *Torreya*, *Juniperus* and *Taxodium* the cytoplasm of the male cell surrounds the fusion nucleus and takes part in the formation of the embryo, but in most genera, no such cyto-

plasm is visible and the embryo is formed from a rather small portion of the basal region of the egg, quite remote from whatever cytoplasm may have entered the egg with the male nucleus.

On such evidence we could not claim, logically, that cytoplasm does not play an essential part in inheritance, for the egg at its first segmentation contains cytoplasm brought in with the male nucleus, but we believe that the series which we traced in spermatogenesis presages the final elimination of any cytoplasm as a part of the male contribution, and the series could be carried into the angiosperms, where, in some cases, the male contributes only a nucleus without any cytoplasm.

In nearly all the gymnosperms the immediate response to the stimulus of fertilization is a series of nuclear divisions which follow each other in such rapid succession that no cell walls are formed between the nuclei. The divisions are simultaneous, probably because the nuclei are in a common mass of cytoplasm exposed to the same conditions. In the large eggs of the cycads, the free nuclear divisions continue until there may be more than a thousand nuclei, but in forms with smaller eggs, the period of free nuclear division is correspondingly reduced, so that we can select a series of genera which show more than a thousand free nuclei, 256 nuclei, then 32, 16, 8, 4 and finally no free nuclear division at all, the first nuclear division of the fertilized egg being followed by the formation of a wall between the daughter nuclei.

These early stages in the gymnosperm sporophyte are remarkably like the early stages of the gametophyte, which also has a prolonged period of free nuclear division before walls begin to be formed, but the conditions are also very similar. The most striking difference between the sporophyte and gametophyte in these early stages is that during mitosis one shows twice as many chromosomes as the other. Very soon, of course, the two generations become very dissimilar. It is worth recalling, in this connection, that in some algæ, like

Dictyota and *Polysiphonia*, the two generations remain similar throughout the vegetative period, the only distinguishing feature being the number of chromosomes.

In conclusion, we believe that fertilization is a phenomenon of fundamental importance, and that future investigation dealing especially with the differences between the various chromosomes, differences which may be only fortuitous but which may be constant and important, may throw light upon the problems of variation and heredity. That the fusion of gametes always gives rise to a sporophytic generation and necessitates a reduction of chromosomes somewhere in the life history is not so speculative and the claim is readily admitted for plants above the thallophytes. We believe that it holds even for thallophytes.

In the simplest bryophytes, alternation is already too thoroughly established to throw any light upon the origin of the phenomenon, and the same may well be said of algæ like *Dictyota, Cutleria* and *Polysiphonia*. We believe that even where the first division of the zygote or fertilized egg shows the reduction division, as in *Coleochæte*, there is a true alternation of generations, although the sporophyte generation is very short. The test of a sporophyte is not its longevity. The fertilized egg of a lily is the first cell of the sporophyte, whether it ever divides at all. Consequently, we regard the zygospore of *Ulothrix* or *Spirogyra* and the fertilized egg of *Vaucheria* or *Œdogonium* as sporophytic structures, even if the first division of the zygote should be meiotic, as seems probable. From such a simple beginning, we believe that the more complex sporophytes with more conspicuous alternation have been developed. The gymnosperms throw no light upon the origin of alternation, but show suggestive stages in the reduction of the gametophytes. They also afford an admirable field for the study of some aspects of fertilization, but we can hardly claim that all the problems of this complex phenomenon would be solved with greatest certainty by the study of cycads or pines.